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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/573,813	07/27/2006	Mitsufumi Wada	1034232-000019	9341

21839 7590 10/21/2011

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ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
10/21/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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Continuation sheet

Continuation of 3. NOTE: Amended claim 7 introduces specific limitation, i.e., "wherein said *Escherichia coli*'s FAD-dependent D-lactate dehydrogenase (dld) inherent activity is inactivated". None of the claims previously examined recited "wherein said *Escherichia coli's* FAD-dependent D-lactate dehydrogenase (dld) inherent activity is inactivated". This limitation was not previously examined requiring new search and consideration of the art made of record, and of the specification for support of the amendment. Addtionally, the scope of claim 7 has been narrowed to recite "wherein said [*Escherichia coli's*] NADH-dependent D-lactate dehydrogenase activity is enhanced". This limitation was not previously examined requiring new search and consideration of the art made of record, and of the specification for support of the amendment. Therefore, the amendment to the claims filed on 10-11-2011 has not been entered.

Continuation of 11. does NOT place the application in condition for allowance because Applicants' arguments rely upon and are directed to the proposed amendments. As the claims' amendment has not been entered, applicants' arguments based on the proposed amendment are not persuasive. Therefore, the rejections of record are maintained.

Rejections maintained in response to Applicants' arguments

Claims 7, 15, 16, 18, 42 and 44 remain rejected under 35 USC 103 as being unpatentable over Zhou et al., (Jan. 2003, Applied and Environmental Microbiology, pp. 399-407) in view of Yang et al., (1999, Metabolic Engineering, pp. 141-152), and further in view of Shaw et al., (1975, J. Bacteriology, pp. 1047-1975).

Reply to applicants' arguments as they relate to rejection of claims 7, 15, 16, 18, 42 and 44 under 35 USC § 103

At page 11-16 of Applicants' response filed on 10-11-2011, Applicants essentially argue that: 1) Fig. 4 of Yang indicates that the yield of acetate from the mutants having enhanced *ldhA* activity (pTY11 and pTY2) increases, as compared with control pACYC184 which leads to the assertion by Yang that an increase in the LTD activity not only increases its own flux and the common flux, but also increases the flux of the competing branch, 2) Yang states " These results

are quite different from a branch point with normal enzyme kinetics, where a flux amplification in one branch often leads to a decrease in the competing branch", 3) Table 23 in Applicant' specification evidences that "the amount of acetic acid from the mutant having enhanced *IdhA* activity, Strain E, increases to 7.0 g/L as compared with Strain C, which has normal *IdhA* activity and 4.4 g/L acetic acid...Yet, surprisingly, Strain F, having enhanced *IdhA* with deleted did activity, has a reduced amount of acetic acid - 4.2 g/L compared with 4.4 g/L for Strain C. Thus, Strain F contradicts Yang's teaching that a mutant with enhanced *IdhA* activity will have increased acetic acid yield". The above arguments have been fully considered but deemed unpersuasive.

Regarding 1) and 2), the fact that overexpression of lactate dehydrogenase (*ldhA*) with an increase of 10 times in the LDH activity fails to divert a large fraction of the carbon flux to lactate probably by activation of the pyruvate formate lyase (PFL) , which leads to formation of formate and acetyl-CoA as disclosed by Yang (abstract) is not disputed. Note that Yang et al., unambiguously discloses that expression of pTY11 and pTY2 in transformant *E. coli* (e.g, plasmid carrying the *ldhA* gene exhibited higher LDH activity, e.g., 25.1, vs. control values of 0.63 (Yang , p. 143, Table 3). As previously stated, the instant claims are product claims which do not require overexpression of a lactate dehydrogenase (*ldhA*) gene for increased of LDH activity, but merely require that the isolated microorganism comprises the fermentative NADH-dependent D-lactate dehydrogenase gene which enzyme is clearly disclosed by both Zhou et al., and Yang et al., in *E. coli* as a critical enzyme for the production of D-lactate, a FAD-dependent D- lactate dehydrogenase (*dld*) activity which catalyzes mainly the reverse reaction from D-lactic acid to pyruvic acid is inactivated or decreased and the pyruvate formate-lyase (*pfl*) inherent in the microorganism which is present in the route of the formation of acetic acid and formic acid is inactivated or decreased. Note that the instant claims do not even require the *ldhA* gene from *E. coli* to have any activity.

Regarding 3), Table 23 discloses that MG1655ΔpflΔdld/pGAPldhA strain as related to MG1655Δpfl/pGAPldhA exhibited enhanced production of D-lactic acid (65 g/L vs 43 g/L) and reduced production of pyruvic acid and acetic acid (0.7 g/L vs. 0.9 g/L; 4.2 g/L vs. 7.0 g/L) which clearly indicates that MG1655ΔpflΔdld/pGAPldhA strain is more effective in the production of D-lactic acid that MG1655Δpfl/pGAPldhA. However, applicants' argument that,

"Table 23 in Applicant' specification evidences that "the amount of acetic acid from the mutant having enhanced IdhA activity, Strain E, increases to 7.0 g/L as compared with Strain C, which has normal IdhA activity and 4.4 g/L acetic acid…Yet, surprisingly, Strain F, having enhanced IdhA with deleted did activity, has a reduced amount of acetic acid - 4.2 g/L compared with 4.4 g/L for Strain C" is not found persuasive because it is noted that the features upon which applicant relies (i.e., enhanced production of D-lactic acid production and reduction of pyruvic acid and acetic acid) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See In re Van Geuns, 988 F.2d 1181, 26USPQ2d 1057 (Fed. Cir. 1993). This is the case here. The claims do not recite isolated E. coli MG1655ΔpflΔdld/pGAPldhA strain with D-lactic production taught in the specification. Furthermore, Applicants' position of surprisingly results for Strain F insofar as having enhanced *ldhA* activity with deleted *dld* and *pfl* genes is not persuasive because Applicants' opinion is unsupported by any specific or real evidence, while the options of the skill in the art are given respectful consideration, in the absence of any actual evidence of "unexpected results", the opinions of the inventor do not overcome a case of *prima facie* obviousness. The arguments of counsel cannot take the place of evidence in the record. *In re Schulze*, 346 F.2d 600, 602, 145 USPQ 716, 718 (CCPA 1965); *In re Geisler*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997). See **MPEP § 716.01(c)** for examples of attorney statements which are not evidence and which must be supported by an appropriate affidavit or declaration. Examples of attorney statements which are not evidence and which must be supported by an appropriate affidavit or declaration include statements regarding unexpected results, commercial success, solution of a long-felt need, inoperability of the prior art, invention before the date of the reference, and allegations that the author(s) of the prior art derived the disclosed subject matter from the applicant. MPEP 716.01(c).

Claims 41, 43 and 45 remain rejected under 35 USC 103 as being unpatentable over Zhou et al., (Jan. 2003, Applied and Environmental Microbiology, pp. 399-407, or record) in view of Yang et al., (1999, Metabolic Engineering, pp. 141-152 , of record) and Shaw et al., (1975, J. Bacteriology, pp. 1047-1975) as applied to claims 7, 15, 16, 18, 42 and 44 above, and further in view of Courtright et al., (*J Bacteriol.* 1970, pp. 722–728, of record).

The Examiner refers Applicants to the reasons already of record, as disclosed in the previous office action of 07/11/2011 at pages 11 and 12, and the reasons set forth in the paragraph above, particularly, to fact that the instant claims are not limited to a mutant *E. Coli* wherein the activity of pyruvate formate-lyase (*pfl*) inherent in the microorganism which is present in the route of the formation of acetic acid and formic acid is inactivated, the FAD-dependent D- lactate dehydrogenase (*dld*) activity which catalyzes mainly the reverse reaction from D-lactic acid to pyruvic acid is inactivated and the LDH activities of strain carrying plasmid pGAPldhA with a higher LDH activity, wherein said mutant *E. Coli* exhibits increased D-lactic acid production (e.g., MG1655ΔpflΔdld/pGAPldhA strain and MG1655ΔpflΔdld/ GAPpldh genome-inserted strain as related to MG1655ΔpflΔdld strain wherein insertion of the GAPpldh genome appears to enhance production of D-lactic acid after 20 hr incubation as related to MG1655ΔpflΔdld/pGAPldhA strain and MG1655ΔpflΔdld strain (paragraph [0087] of the published application]).

Claim 18 and 19 remain rejected under 35 USC 103 as being unpatentable over Zhou et al., (Jan. 2003, Applied and Environmental Microbiology, pp. 399-407, or record) in view of Yang et al., (1999, Metabolic Engineering, pp. 141-152 , of record) and Shaw et al., (1975, J. Bacteriology, pp. 1047-1975) as applied to claims 7, 15, 16, 18, 42 and 44 above, and further in view of Maier et al (US Patent Application No. 10/620487, Date of filing July 16, 2003)

The Examiner refers Applicants to the reasons already of record, as disclosed in the previous office action of 07/11/2011 at pages 12 and 13, and the reasons set forth in the paragraph above, particularly, to fact that the instant claims are not limited to a mutant *E. Coli* wherein the activity of pyruvate formate-lyase (*pfl*) inherent in the microorganism which is present in the route of the formation of acetic acid and formic acid is inactivated, the FAD-dependent D- lactate dehydrogenase (*dld*) activity which catalyzes mainly the reverse reaction from D-lactic acid to pyruvic acid is inactivated and the LDH activities of strain carrying plasmid pGAPldhA with a higher LDH activity, wherein said mutant *E. Coli* exhibits increased D-lactic acid production (e.g., MG1655ΔpflΔdld/pGAPldhA strain and MG1655ΔpflΔdld/ GAPpldh genome-inserted strain as related to MG1655ΔpflΔdld strain wherein insertion of the GAPpldh genome appears to enhance production of D-lactic acid after 20 hr incubation as

related to MG1655ΔpflΔdld/pGAPldhA strain and MG1655ΔpflΔdld strain (paragraph [0087] of the published application]).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Maria Leavitt/

Maria Leavitt
Primary Examiner, Art Unit 1633

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furthermore, the claimed isolated microorganism does place any limitation on the intended use such as high levels of production of D-lactate with concomitant reduction of pyruvic acid, for example, as disclosed at page 43, Table 7 of the specification as filed.

and

hough Zhou describes an *E. coli* mutant having a deleted *pfl* which exhibits increased production of D-lactic acid in relation to parental strain, and Yang teaches that increased LDH activities of *E. coli* strains carrying the *ldhA* plasmid produced higher D-lactic acid relative to control strain, in part by elimination of competing pathways for the production of D-lactate and metabolizing pyruvate to D-lactic acid, there is not sufficient reason to combine the teachings of Zhou and Yang as a whole, because Yang itself indicates that the Examiner's assertions are not scientifically-sound by stating: